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# High-resolution ice nucleation spectra of sea-ice bacteria: implications for cloud formation and life in frozen environments

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**BGD**

4, 4261–4282, 2007

**Ice nucleation by  
sea-ice bacteria**

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

## Abstract

Even though studies of Arctic ice forming particles suggest that a bacterial or viral source derived from open leads could be important for cloud formation in the Arctic (Bigg and Leck, 2001), the ice nucleation potential of most polar marine psychrophiles or viruses has not been examined under conditions more closely resembling those in the atmosphere. In this paper, we examined the ice nucleation activity (INA) of several representative Arctic and Antarctic sea-ice bacterial isolates and a polar *Colwellia* phage virus. High-resolution ice nucleation spectra were obtained for droplets containing bacterial cells or virus particles using a free-fall freezing tube technique. The fraction of frozen droplets at a particular droplet temperature was determined by measuring the depolarized light scattering intensity from solution droplets in free-fall. Our experiments revealed that all sea-ice isolates and the virus nucleated ice at temperatures very close to the homogeneous nucleation temperature for the nucleation medium – which for artificial seawater was  $-42.2 \pm 0.3^\circ\text{C}$ . Our results indicated that these marine psychro-active bacteria and viruses are not important for heterogeneous ice nucleation processes in sea ice or polar clouds. These results also suggested that avoidance of ice formation in close proximity to cell surfaces might be one of the cold-adaptation and survival strategies for sea-ice bacteria. The fact that INA occurs at such low temperature could constitute one factor that explains the persistence of metabolic activities at temperatures far below the freezing point of seawater.

## 1 Introduction

Psychro-active (including both psychrotolerant and psychrophilic) bacteria are capable of growing at temperatures at least as low as  $-1^\circ\text{C}$  and are important constituents of polar ecosystems. These bacteria have successfully evolved genotypic and phenotypic features to surmount the negative effects of low temperatures on cell functions thereby enabling them to remain active in extreme low temperature environments (Deming,

**BGD**

4, 4261–4282, 2007

### Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

2002; D'Amiko et al., 2006; Junge et al., 2006). In this paper, we investigated the ice initiation potential of several representative sea-ice bacterial isolates to assess the role that marine psychro-active bacteria could play in the formation of polar clouds and of sea ice. These results may also provide a basis for exploring possible mechanisms for psychrophilic adaptation to ice crystal formation.

The climate of the earth is very sensitive to the microphysical, radiative and chemical properties of glaciated clouds (Houghton et al., 2001; Vogelmann and Ackerman, 1995). Accurate climate modeling requires that the entire process from particle formation to cloud drop nucleation be known (Bigg and Leck, 2001). Studies of ice formation in Arctic clouds have suggested that marine bacteria and other particles of biological origin derived from open leads within the sea-ice cover could be important for cloud formation in the Arctic (Bigg and Leck, 2001), but few experiments have measured the ice nucleation activity of polar marine bacteria (Parker et al., 1985).

Bacteria found trapped within sea-ice could be involved in its nucleation (summarized by Sullivan, 1985). Sea ice is known to be of great importance to polar and oceanic climate (Eicken and Lemke, 2001) but the origin, composition, and number of ice nuclei that may initiate the formation of sea ice are still not well understood. At the beginning of winter marine psychrophilic INA (i.e. ice nucleation active) bacteria could provide such a source when lifted into the air and fallen back into the water. To date, there is some evidence for such an involvement of sea-ice bacteria in its formation (summarized by Sullivan et al., 1985), including one report of an unidentified sea-ice bacterial strain to be ice nucleation active at temperatures between  $-2.0$  and  $-3.5^{\circ}\text{C}$  (Parker et al., 1985). *Pseudomonas antarctica*, isolated from Antarctic soil, has also been found to possess INA ( $T_f$ , defined as the freezing temperature where 50% of droplets are frozen, was  $\sim -4^{\circ}\text{C}$  under some incubation conditions, Obota et al., 1999). However, none of these studies were performed using a substrate-free methodology thereby more closely resembling conditions in the atmosphere nor were multiple strains of representative groups of sea-ice bacteria commonly found in these regions tested.

## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

2 Experimental method

2.1 Ice nucleation activity assay

We used a freezing-tube technique (described more fully in Wood et al., 2002; Larson and Swanson, 2006; Swanson, 2007<sup>1</sup>) to assay the freezing spectra of 17 isolates of marine psychro-active bacteria and virus particles. The freezing tube consists of a vertical hollow brass cylinder with long vertical multi-pane windows on opposing sides for illumination and observation of the droplets as they fall. A droplet-on-demand droplet generator is mounted co-axially on top of the tube with a vertical temperature gradient along the tube axis maintained by circulating low-temperature methanol through copper coils attached to the tube base. Droplets are ejected from the droplet-on-demand droplet generator and the droplets cool as they fall down the tube axis (see Fig. 1).

The phase of the droplets (whether they are liquid or solid) at various heights (temperatures) in the tube was determined using a depolarized laser light scattering technique consisting of two video cameras with telemicroscopic lenses, a polarizing filter, and a beam-splitter mounted on a metal stage which, after alignment, may be translated up and down along the freezing tube’s vertical axis (see Fig. 1). To determine the fraction of frozen droplets at a particular temperature, we positioned the phase detection system such that a polarized HeNe laser beam intersected the droplet stream at the height of interest. Using the two cameras (one without and one with a polarizer) viewing the droplet stream through the beam-splitter, we obtained streak-images of both the total, TLS, and depolarized, DLS, light scattered from each falling droplet. We used the fact that light scattered from liquid droplets remains polarized in the laser’s original plane of polarization, whereas some backscattered light from frozen droplets will be depolarized due to droplet asphericity, cracks, bumps, surface roughness and

<sup>1</sup>Swanson, B. D.: Laboratory measurements of the homogeneous freezing of aqueous sulfuric acid and ammonium sulfate droplets: How well does the translated melting-point curve model work?, J. Atmos. Sci., submitted, 2007

Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

birefringence. At a particular height in the freezing tube it is usually evident when the majority of droplets are liquid or frozen by simply looking at the video screen to see whether streaks can be seen only in the TSL image (liquid) or in both the TSL and DSL images (solid). Frozen fraction curves,  $F(T)$ , (the fraction of droplets frozen at temperature  $T$ ) were measured by translating the phase detection system (initially down to where 100% of the droplets are frozen) and then making measurements at intervals between 100% and 0% frozen fraction. At each temperature (height) about 100 to 400 images of droplet streaks were captured on video tape. We later analyzed each streak image by first subtracting the background intensity and then calculating the ratio DLS/TLS for each droplet. Those particles with DLS/TLS above some threshold value (typically about 7% of full-scale intensity) were defined to be frozen and those below that threshold, liquid. We set this threshold signal by comparing the various data sets and selecting a value that required the droplets to be all liquid at high temperature and all solid at temperatures far below the nucleation temperature (essentially at the lowest temperatures at the bottom of the freezing tube). Typically DLS/TLS measurements were made for 3000 to 4000 droplets for a single  $F(T)$ . For the experiments reported here, 15 to 25  $\mu\text{m}$  radius droplets were used with droplets emitted from the droplet generator at a rate of about 5 Hz (to avoid droplet-droplet interactions).

For each data run, the appropriate concentration of bacterial solution was injected into a new (and flushed with HPLC-grade water) droplet generator cartridge and immediately placed atop the freezing tube inside a sealed airtight enclosure. The enclosure and freezing tube was then purged of aerosol-laden room air using dry nitrogen gas thus eliminating to near-zero the potential problem of an occasional influx of aerosol particles inducing droplet freezing through contact nucleation, especially at lower temperatures. Freezing tube temperatures were regularly recorded via computer using thermistors embedded in the cylinder walls and a thermistor attached to the bottom of a movable glass rod which could be positioned at any level vertically in the air within a few mm of the droplet stream.

The droplet temperature at each height in the droplet stream was calculated using a

**BGD**

4, 4261–4282, 2007

## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

computer model of the freezing tube temperature profile where the model inputs were the tube wall temperatures and the chamber humidity profile (obtained from strobe images of the droplets along the stream). This model corrects for temperature lag or lead due to droplet evaporation/growth or fall-speed effects. For the results shown here the droplet sizes were quite stable throughout their fall and no temperature correction was required. Typically, the wall temperature changed no more than a few hundredths of a degree every hour. Further discussion of the analysis and experimental details can be found in previous publications (Wood et al., 2002; Larson and Swanson, 2005; Swanson, 2007<sup>1</sup>).

## 2.2 Sample preparation

All bacterial strains tested for INA are listed in Table 1. The Arctic sea-ice bacterial strains were isolated from ice-core samples collected during the Arctic West Summer 1996 (AWS96) cruise of the USCGC icebreaker “Polar Sea” into the Chukchi Sea (Table 1; Junge et al., 2002). *C. psychrerythraea* str. 34H was isolated from Arctic shelf sediments (Huston et al., 2000). *Planococcus mcmeekeni* str. S23F1 was isolated from Antarctic sea ice (Junge et al., 1998). Polar bacterial cultures were grown at  $-1^{\circ}\text{C}$  for 14 days (to late exponential growth) in half-strength Marine 2216 broth (DIFCO laboratories, Detroit, MI). Bacterial cells were then centrifuged at  $1500 \times g$  for 5 min, washed twice in artificial seawater (ASW; for 1 l of solution: 24 g NaCl, 0.7 g KCl, 5.3 g  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 7.0 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 1.3 g TAPSO buffer), and after the final centrifugation step were re-suspended into artificial seawater to yield a final optical density at 600 nm (OD) of 0.5. The cold-active *Colwellia* phage V-9 virus was kindly provided by Llyd Wells (Wells and Deming, 2006) at a concentration of approximately  $10^9$  virus particles  $\times \text{ml}^{-1}$  in phage lysis buffer (0.1 M NaCl, 50 mM Tris-HCl pH 8.0, 8 mM  $\text{MgSO}_4$ , 2 mM  $\text{CaCl}_2$  and 5% glycerol). Cultures of *Pseudomonas syringae* str. R31 were kindly provided by Steve Lindow’s laboratory. Fresh cultures of *Pseudomonas syringae* str. R31 were grown at room temperature for 3 days on Nutrient Agar plates. Cells were then harvested from plates, suspended in sterile distilled water, centrifuged at  $1500 \times$

### Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

g for 5 min, washed twice in sterile distilled water and after the final centrifugation step were re-suspended into distilled water or ASW to yield a final optical density at 600 nm of 2.0.

Generally, ice nucleation tests were performed by adjusting the optical density of polar bacterial suspensions in sterile artificial seawater at 600 nm to 0.5. The temperature-dependent freezing rates were then determined from each isolate, the virus preparation and artificial seawater (ASW). We used droplets of 0.10 nl volume. To ensure that each droplet contained at least one bacterium at the optical density used, we determined the concentration of bacterial cells in our ASW suspensions for one of the isolates (*Colwellia psychrerythraea* str. 34H) by counting the number of bacteria in collections of frozen droplets emitted from the droplet generator (see below) using standard epifluorescence microscopy and the DNA-specific stain 4',6'-diamidino-2-phenylindole 2HCl (DAPI), essentially as described by Junge et al. (2002). Determinations of bacterial concentration in collections of ejected droplets revealed that 3 to 5 bacteria were present in each droplet thus confirming that enough potential nuclei were present to facilitate ice nucleation.

### 3 Results and discussion

High-resolution ice nucleation spectra (INA) of selected sea-ice bacterial isolates that were representatives for most of the major known groups of sea-ice bacteria (both of Antarctic and Arctic origin) that we tested are shown in Fig. 2 and Fig. 3 in comparison to the artificial seawater (ASW) nucleation medium. The freezing temperature,  $T_f$ , was defined to be the temperature where  $F(T)=50\%$ . Our tests revealed that sea-ice isolates and phage virus particles nucleated ice at temperatures very close to the homogeneous nucleation temperature for the nucleation medium ( $T_f$  for ASW:  $-42.2\pm0.3^\circ\text{C}$ , see Table 1). Thus, the average  $T_f$  ( $\pm$  standard deviation) for the psychro-active bacterial isolates was found to be  $-41.2\pm0.^\circ\text{C}$  (range  $-40.4$  to  $-42.7^\circ\text{C}$ ,  $n=15$ ). For *P. syringae* str. R31 suspended in distilled water at OD of 2.0, we found ice nucleation

## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



temperatures that are in good agreement with published values ( $T_f = -6.3^\circ\text{C}$ , Lindow, 1985).

These results suggest that the sea-ice bacteria and viruses tested here have a very limited nucleation activity. Our finding stands in contrast to earlier suggestions that such bacteria are involved in polar cloud precipitation processes (Bigg and Leck, 2001). Other species, however, derived from other sources, such as vegetation derived *P. syringae*, also shown to occur in the Arctic atmosphere (Jayaweera and Flanagan, 1982) and/or *P. antarctica*, derived from Antarctic soil (Obota et al., 1999) or Antarctic lake bacteria (Gilbert et al., 2004) could provide alternative sources for ice forming nuclei (IFN) in the atmosphere, but *direct* (in situ) evidence for this activity and bacterial activity in the atmosphere in general remains to be demonstrated (for review see Szyrmer and Zawadski, 1997; Möhler et al., 2007). Obtaining such knowledge is especially important as metabolic activity and intact proteins of the outer membrane appear to be required for effective ice nucleating property (Hazra et al., 2004).

So what role do bacteria play in the polar atmosphere? Knowledge of bacterial diversity and activity in the atmosphere is scarce, especially for the Polar region (Carpenter et al., 2000; Posfai et al., 2003; Amato et al., 2007; Elster et al., 2007) and has recently become of greater research interest (see references in Sattler et al., 2001; Bauer et al., 2002; Ahern et al., 2006; Möhler et al., 2007). In Antarctica, devoid of a terrestrial source for ice nuclei (IN), studies suggest that biological nuclei play a role in the formation of coastal clouds and that the ocean surface might be their source (Saxena, 1983). Schnell (1975) proposed that marine organisms may be responsible for the bands of airborne ice nucleation activity found along latitudes of  $40^\circ$  and  $55^\circ$  South by Bigg (1973). Schnell and Vali (1976) found that seawater collected in Bedford Basin, Nova Scotia were good sources of IN when they contained phytoplankton with some IN active at  $-4^\circ\text{C}$ . Our findings are in stark contrast to these results and this could be due to two causes: Substrate-based methods for measuring low-temperature INA can be problematic if substrates are not sufficiently well passivated. The freezing tube method has been tested by measuring the homogeneous ice nucle-

**BGD**

4, 4261–4282, 2007

## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

ation temperature of aqueous sulfuric acid and ammonium sulfate solutions (Larson and Swanson, 2006 and Swanson, 2007<sup>1</sup>). Considerable effort has been made to reduce to near zero (by sample filtration and flushing sample dispensers and the freezing tube etc.) the probability of the occurrence of other heterogeneous processes. Sample droplets are not in contact with substrates in this method and the lower temperature limit (about  $-65^{\circ}\text{C}$ ) is set by the cooling capacity of the cryogenic bath unit. To check this method, the homogeneous freezing temperature of pure water was measured to be  $-37^{\circ}\text{C}$  – which is consistent with previous experiments (see Wood et al., 2002). We considered marine psychrophiles to be good candidates for high-temperature INA, since they are abundant in polar waters and sea-ice but seawater samples also contain other potential IN such as phytoplankton, viruses, fungi, dust, salts etc. Thus the low-temperature INA observed for our sea-ice bacterial isolates would be masked by the presence of higher-temperature IN in, for instance, seawater samples. Perhaps other IN referred to as ocean-derived nuclei (ODN) are responsible for the nucleation observed by Schnell and Vali (1976). The transfer of materials from the oceans to the atmosphere is well documented (Schnell and Vali, 1976). These materials, when lifted into the air in regions of an active sea-to-air transfer mechanism (demonstrated to occur through the bursting of bubbles (Schnell and Vali, 1976; Szyrmer and Zawadzki, 1997), could cause nucleation and thus affect polar cloud processes. Future experiments will explore other materials and additional sea-ice bacterial isolates to identify the species responsible for the observed higher-temperature INA. It should be noted that, except for the ODN described by Schnell and Vali (1976), the world oceans have not been found to be a source of IN with many studies demonstrating that maritime air masses are consistently deficient in IN (Pruppacher and Klett, 1997). The limited polar bacterial ice nucleation activity documented here adds to evidence of undetectable bacterial ice nucleation activity found by others in cloud and rain samples collected from Scottish mountains tops (Ahern et al., 2006). Using culture-independent molecular methods, Ahern et al. (2006) determined that cloud bacteria were closely related to bacteria from other cold environments and were dominated by a mixture of fluorescent

**BGD**

4, 4261–4282, 2007

## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

*Pseudomonas* spp. (thought to be efficient ice nucleators), though with no detectable ice nucleation genes. Instead the majority of isolates displayed significant biosurfactant activity. Though it is too early to draw general conclusions based on only two studies, these findings cause us to formulate the hypothesis that the majority of airborne bacteria are not ice nucleation active but rather attract available liquid water sources (by producing exopolymeric substances (EPS) as many sea-ice bacteria (Krembs et al., 2002) or biosurfactants and thus becoming cloud condensation nuclei (CCN) active (as demonstrated by Bauer et al., 2002 and Ahern et al., 2006). This would allow for water scavenging, countering desiccation, and assist in their widespread dispersal (Ahern et al., 2006). In other non-polar regions, bacterial ice nucleation activity has been so far extensively investigated only in terrestrial plant bacteria such as *Pseudomonas syringae* (see for review Lee et al., 1995). There is no clear evidence however that they play a role in the formation of ice in clouds (for review see Möhler et al., 2007). Plant-derived bacteria have been observed in the atmosphere above plant canopies at heights up to 6 km (Lindemann et al., 1982; Lindemann and Upper, 1985) and in raindrops, hailstones both in North America, Europe, Asia and the Arctic (Lindow et al., 1978; Yankofsky et al., 1981; Jayaweera and Flanagan, 1982), however, attempts to obtain typical marine microorganisms with INA have not been met with success, with the exception of one INA bacterial strain phenotypically very similar to *Pseudomonas fluorescence* isolated from a marine dinoflagellate culture (Fall and Schnell, 1985).

Our results indicate that the IN in this population have an INA too low to be involved with sea-ice formation. An earlier study that showed one sea-ice strain (to date still unidentified) to have INA at higher temperatures that suggested such a possible role of sea-ice bacteria (Parker et al., 1985) could not be substantiated using our novel technique and analyzing representatives of most major groups of sea-ice bacteria. Our results though expand and corroborate earlier findings of others who failed to find significant INA in the majority of marine (but not polar) bacteria that were studied (Fall and Schnell, 1985). The present study thus substantiates these earlier observations that were limited because (1) they investigated only a small number of droplets (<100

**BGD**

4, 4261–4282, 2007

## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

droplets as opposed to 2000 to 4000 droplets as in the present study) and (2) they used substrate-based freezing methodologies.

Ice is ubiquitous, not only on Earth, but also in our solar system. Our examination of the freezing behavior of polar ice bacteria revealed how these bacteria might cope with freezing temperatures and the ice itself – by largely avoiding ice nucleation and thereby remaining within liquid water, essential prerequisite to maintain growth. Sea ice is the coldest marine habitat on Earth and the mechanism for survival of polar marine bacteria during cold and salty conditions are of considerable interest. Sea-ice liquid brine temperatures can reach as low as  $-35^{\circ}\text{C}$  with corresponding in situ salinities of 240 (Cox and Weeks, 1983; Maykut, 1986). These conditions are known to select for psychrophiles (Helmke and Weyland, 1995; Bowman et al., 1997; Nichols et al., 1999; Bowman et al., 2005) and sea-ice bacteria are model organisms to study polar marine psychrophily. Such bacteria have been shown to be active in their environment down to  $-20^{\circ}\text{C}$  and 209 ppt (Junge et al., 2004 and possibly even much lower (to  $-196^{\circ}\text{C}$ ) see Junge et al., 2006) and have developed strategies to overcome detrimental effects of very cold temperatures well-known for a wide range of bacterial processes (for a review see Deming 2002). Much published research on sea-ice organisms has focused on their diversity (reviewed by Bowman et al., 2005), enzyme and membrane adaptations (reviewed by Nichols et al., 1999; Deming, 2002) and field observations of sea-ice microbial communities (e.g. Helmke and Weyland, 1995; Junge et al., 2004). During the freezing processes itself the majority of bacteria apparently remain within liquid brine pockets within the ice matrix (Junge et al., 2001) possibly by reducing the close proximity of ice to the outer cell wall during the passing of a freezing front. Survival during ice crystal formation or once encased in sea ice certainly also involves the avoidance of ice crystal formation within their cells (Deming, 2002; D’Amico et al., 2006). Having limited ice nucleation activity could be a bacterial strategy for avoiding direct attachment to ice and thus also reducing the likelihood of ice crystal formation within their cells.

The apparent avoidance of INA raised the following question: Do sea-ice bacte-

Ice nucleation by  
sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

ria exhibit special ice preventative surface structures such that they largely avoid the ice initiation that other solid objects of their size would experience? An examination of the surface of some of our sea-ice isolates using surface-enhanced Raman-spectroscopy techniques (Laucks et al., 2005) indicated that compared to other gram-negative mesophilic bacteria psychro-active isolates do possess unique surface structures, likely represented by their different membrane fatty acid composition. Within the sea-ice environment, our results suggest that sea-ice bacteria as they represent ice-forming nuclei with INA at sufficiently low temperatures will largely avoid ice formation around their cells. Thus they remain in their liquid brine environment (as opposed to being frozen directly into the solid ice) with nutrient exchange and metabolic activity possible to the lowest subzero temperatures encountered there ( $-35^{\circ}\text{C}$ ).

## 4 Summary

We have used the freezing tube methodology to observe the INA of sea ice bacteria isolates away from the possible effects of substrates. Studying INA away from substrate surfaces is critical since prior to this study, most bacterial ice nucleation studies have been performed on solid surfaces (with a few exceptions, Möhler et al., 2007). We find low-temperature INA for the isolates observed in this study and these isolates are therefore not likely to be important for polar atmospheric processes. Overall, knowledge of bacterial diversity and activity is essential to predict potential bacterial impact on cloud formation processes and air chemistry if metabolically active bacteria process organic and inorganic species of importance for atmospheric processes (Ariya and Amyot, 2004). We conclude that the degree of ice nucleation activity derived through bacteria in polar clouds (either limited ice nucleation activity, e.g. by sea-ice bacteria, or high ice nucleation activity (e.g. by *P. syringae* or *P. antarctica* – type bacteria) will depend on the bacterial species composition (diversity) and bacterial activity in the atmosphere.

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## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

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- 30

**BGD**

4, 4261–4282, 2007

## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

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**BGD**

4, 4261–4282, 2007

## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**



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K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

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**BGD**

4, 4261–4282, 2007

---

## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

---

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

**EGU**

**Table 1.** List of sea-ice and other bacterial isolates tested for INA, their closest relatives, habitat and  $T_f$  -defined as the temperature at which 50% of the droplets are frozen.

Isolate	Species or closest relative	Phylum	Habitat	$T_f$ *
34H	<i>Colwellia psychrethraea</i> str. 34H	$\gamma$ -Proteobacteria	Arctic sediment and sea ice, Antarctic sea ice	$-42.0 \pm 0.5$
6M3	<i>Colwellia</i> sp. IC169	$\gamma$ -Proteobacteria	Arctic and Antarctic sea ice	$-40.9 \pm 0.5$
4U2	Gas-vacuolate strain 214 <sup>b</sup>	$\gamma$ -Proteobacteria	Arctic sea ice	$-40.7 \pm 0.5$
6M4	Gas-vacuolate strain 214 <sup>b</sup>	$\gamma$ -Proteobacteria	Arctic sea ice	$-40.4 \pm 0.2$
6B1	Gas-vacuolate strain 214 <sup>b</sup>	$\gamma$ -Proteobacteria	Arctic sea ice	$-41.1 \pm 0.9$
7M1	<i>Pseudoalteromonas antarctica</i> str.. IC 013	$\gamma$ -Proteobacteria	Arctic and Antarctic sea ice	$-41.4 \pm 0.6$
4M2	<i>Pseudoalteromonas antarctica</i> str.. IC 014	$\gamma$ -Proteobacteria	Arctic and Antarctic sea ice	$-40.6$
11B5	<i>Shewanella frigidimarina</i>	$\gamma$ -Proteobacteria	Arctic and Antarctic sea ice	$-41.1 \pm 0.8$
37P	<i>Psychromonas ingrahamii</i> str. 37P	$\gamma$ -Proteobacteria	Arctic sea ice	$-42.7$
4U1	<i>Cytophaga</i> sp JTB 244	CFB**	Arctic sea ice, Japan Trench	$-41.7$
4M6	marine psychrophile SW 17 <sup>a</sup>	CFB	Arctic sea ice, Antarctic seawater	$-41.3 \pm 0.9$
4M7	<i>Flavobacterium xanthum</i>	CFB	Arctic and Antarctic sea ice	$-41.0 \pm 0.4$

## Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Ice nucleation by  
sea-ice bacteriaK. Junge and  
B. D. Swanson

Table 1. Continued.

Isolate	Species or closest relative	Phylum	Habitat	$T_f$ *
6B2	<i>Polaribacter franzmannii</i>	CFB	Arctic and Antarctic sea ice	$-41.3 \pm 0.6$
4B3	<i>Octadecabacter antarcticus</i>	$\alpha$ -Proteobacteria	Arctic and Antarctic sea ice	$-41.4 \pm 0.5$
S23F1	<i>Planomicrobium mcmeekenii</i>	Low-GC Gram-positives	Antarctic sea ice	$-41.4$
Virus	<i>Colwellia</i> infecting phage V 9		Arctic sediment, Antarctic sea ice	$-41.9^c$
R31	<i>Pseudomonas syringae</i>	$\gamma$ -Proteobacteria	terrestrial, plant leaves	$-6.3^d$
ASW	Artificial seawater			$-42.2 \pm 0.3$
H2O	Distilled water			$-37$
PHB	Phage Buffer			$-42.5$

\*Average +/- standard deviation ( $n=2$ , for ASW  $n=4$ ).\*\* CFB is Cytophaga-Flavobacteria-Bacteroides <sup>a</sup> Closest genus was *Alteromonas*.<sup>b</sup> Closest genus was *Gelidibacter*.<sup>c</sup> Virus in buffer at approx  $10^9$  particles\*ml<sup>-1</sup>.<sup>d</sup> Bacteria in distilled water at optical density of 2.0 at 600 nm.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

I◀

▶I

◀

▶

Back

Close

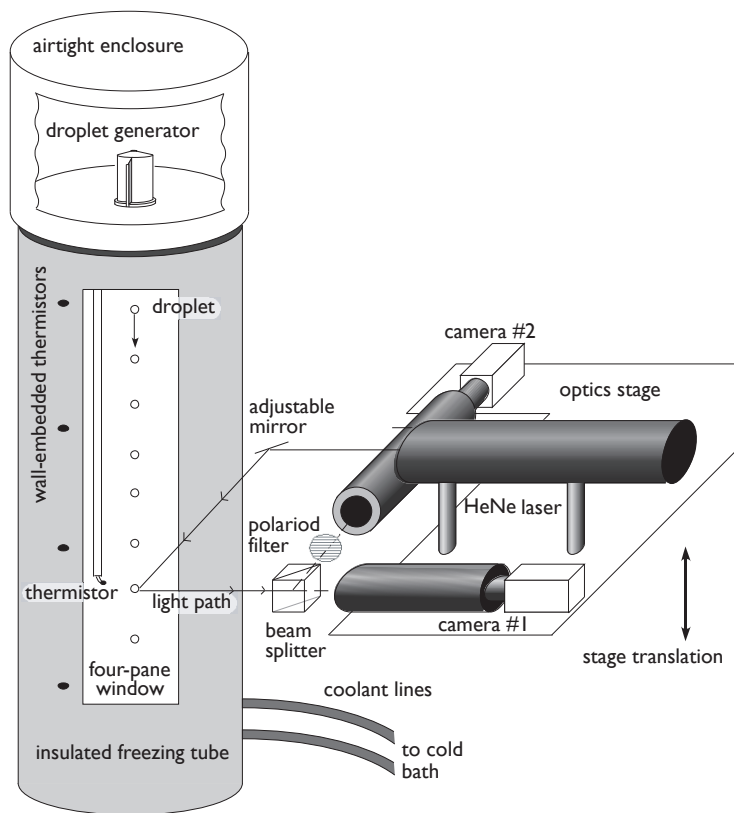
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Printer-friendly Version

Interactive Discussion

# Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson



**Fig. 1.** Schematic representation of freezing tube apparatus (adapted from Larson and Swanson, 2006).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

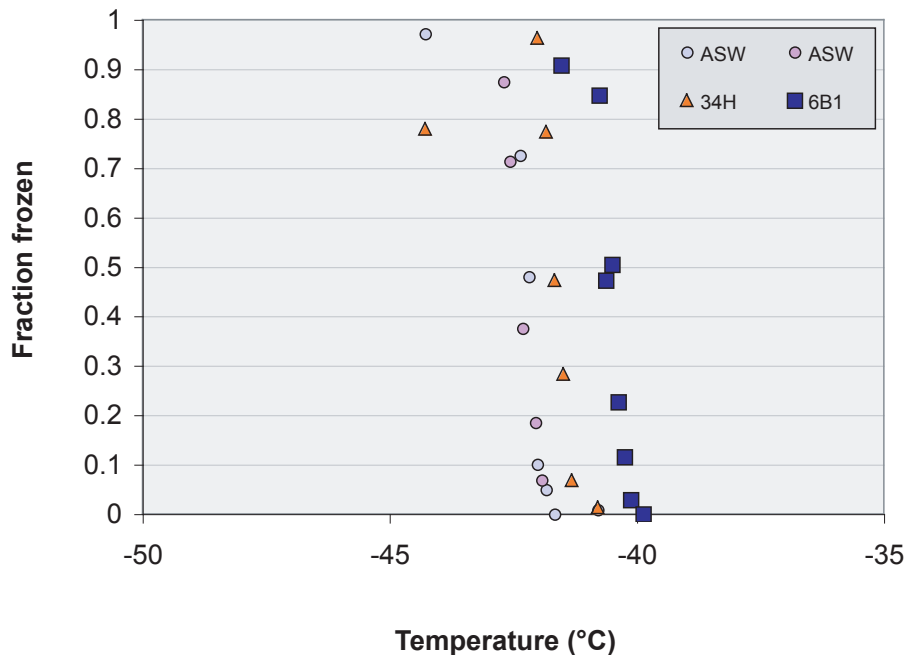
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Printer-friendly Version

Interactive Discussion

# Ice nucleation by sea-ice bacteria

K. Junge and  
B. D. Swanson



**Fig. 2.** Representative freezing spectra for polar bacterial isolates *Colwellia psychrerythraea* str. 34H (triangles), sea-ice strain 6B1 (squares) and ASW (circles).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

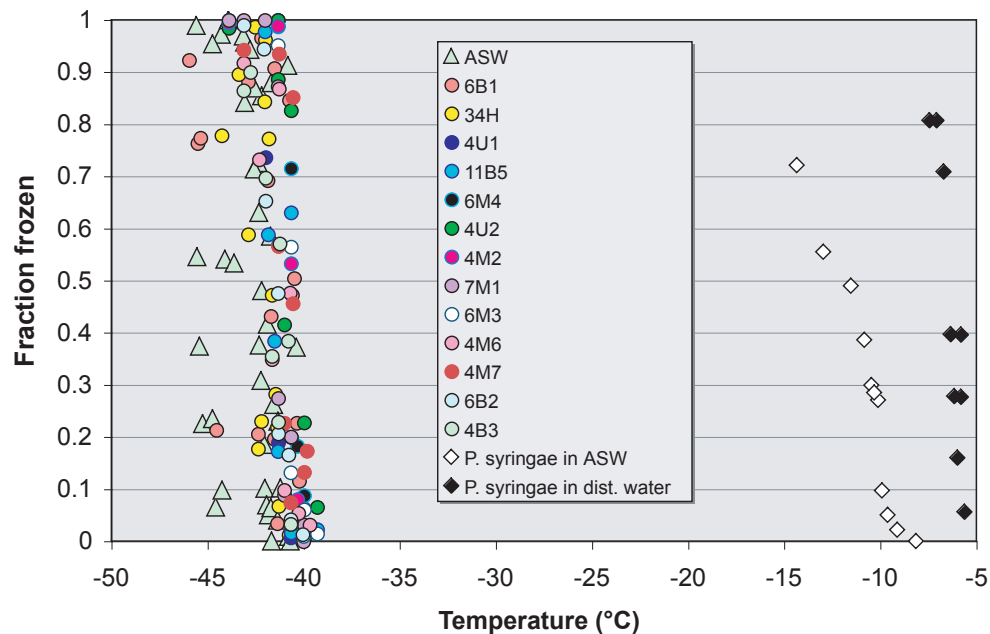
Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Ice nucleation by  
sea-ice bacteriaK. Junge and  
B. D. Swanson

**Fig. 3.** Freezing spectra for ASW (triangles), representative sea-ice bacteria (circles, different colours indicate different strains) in comparison with *P. syringae* (diamonds: black, cells in HPLC-grade water; white, cells in ASW).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion